

What is claimed is:

1. A method for constructing a nucleic acid library comprising:
  - a) obtaining a population of end-labeled double-stranded cDNA molecules;
  - b) dividing said population into a first portion and a second portion;
  - c) digesting said first portion with at least one sequence-specific endonuclease;
  - 5 d) digesting said second portion with at least one endonuclease having a degenerate recognition sequence;
  - e) isolating nucleic acid fragments from said endonuclease digestions of said first and second portions using said end-labels;
  - f) digesting the fragments of said first portion with said at least one endonuclease  
10 of d);
  - g) digesting the fragments of said second portion with said at least one endonuclease of c);
  - h) removing labeled nucleic acid fragments from said first and second portions while retaining unlabeled fragments;
  - 15 i) adding a population of adapters, said population containing adapters specific for the endonucleases used, wherein each adapter comprises a first region specific to a particular endonuclease used and a second region containing a primer binding site;
  - j) hybridizing and ligating said adapters to said unlabeled fragments;
  - k) amplifying the fragments using the adapters; and
  - 20 l) separating the amplified fragments by size.

2. The method of claim 1, wherein said at least one sequence-specific endonuclease comprises at least 4 different sequence specific endonucleases.

3. The method of claim 1, wherein said at least one sequence-specific endonuclease comprises at least 6 different sequence specific endonucleases.

4. The method of claim 1, wherein said at least one sequence-specific endonuclease is selected from the group consisting of at least one endonuclease having a 4 base recognition sequence, at least one endonuclease having a 5 base recognition sequence and at least one endonuclease having a 6 base recognition sequence.

5. The method of claim 3, wherein said at least one sequence-specific endonuclease is selected from the group consisting of *Apa* LI, *Bam* HI, *Eco* RI, *Hind* III, *Nco* I, and *Xho* I.

6. The method of claim 1, wherein said at least one endonuclease having a degenerate recognition sequence produces fragments comprising unpaired overhangs containing  $N^m$  unique sequences where N is the extent of degeneracy and is an integer between 2 and 4, and m is the number of bases in said unpaired overhang and is an integer between 2 and 6.

7. The method of claim 6, wherein  $N^m$  equals at least 64.

8. The method of claim 7 wherein said endonuclease is *Bsl* I.

9. The method of claim 1, further comprising sequencing the separated fragments of 1).

10. The method of claim 1, further comprising quantifying the separated fragments of 1).

11. The method of claim 1, wherein said primer binding region of said adapters does not have significant homology to sequences known to be in said population of nucleic acid molecules.

12. The method of claim 11, wherein said primer binding regions of said adapters comprise no more than 10 different sequences.

13. The method of claim 12, wherein said primer binding region comprise no more than two different sequences.

14. The method of claim 1, wherein said end-labeled nucleic acid molecules are obtained by a method comprising, isolating polyA mRNA from a cell; hybridizing a 5' end labeled an oligo dT primer to said polyA m RNA; synthesizing a first cDNA strand by extension of said primer; and synthesizing a second cDNA strand by nick translation to produce double stranded cDNA.

15. The method of claim 14, wherein said oligo dT primer has a sequence comprising L-(T)<sub>n</sub>VN, where L is a label at the 5' end of said primer and n is an integer between 4 and 50.

16. The method of claim 14, wherein said at least one sequence-specific endonuclease comprises at least 4 different endonucleases.

17. The method of claim 14, wherein said at least one sequence-specific endonuclease is selected from the group consisting of at least one endonuclease having a 4 base recognition sequence, at least one endonuclease having a 5 base recognition sequence and at least one endonuclease having a six base recognition sequence.

18. The method of claim 14, wherein said at least one sequence-specific endonuclease comprises at least 6 different endonucleases.

19. The method of claim 18, wherein said at least one sequence-specific endonuclease is selected from the group consisting of *Apa* LI, *Bam* HI, *Eco* RI, *Hind* III, *Nco* I, and *Xho* I.

20. The method of claim 14, wherein said at least one endonuclease having a degenerate recognition sequence produces fragments comprising unpaired overhangs

containing  $N^m$  unique sequences where  $N$  is the extent of degeneracy and is an integer between 2 and 4, and  $m$  is the number of bases in said unpaired overhang and is an

5 integer between 2 and 6.

21. The method of claim 20, wherein  $N^m$  equals at least 64.

22. The method of claim 21 wherein said endonuclease is *Bst* I.

10

23. The method of claim 14, wherein said label is biotin.

24. The method of claim 14, wherein isolating and removing of end-labeled nucleic acid molecules is by particles that bind said end-labeled nucleic acid molecules.

15

25. The method of claim 24, wherein said end-label is biotin and said particles comprise avidin or streptavidin.

26. The method of claim 14, further comprising sequencing the separated fragments of 1).

20

27. The method of claim 14, further comprising quantifying the separated fragments of 1).

28. A method for constructing a nucleic acid library comprising:

a) obtaining a population of 3' end-labeled double-stranded cDNA molecules by a method comprising, isolating polyA mRNA from a cell; hybridizing a 5' end labeled primer containing an oligo dT tail to said polyA mRNA; synthesizing a first cDNA strand by extension of said primer; and synthesizing a second cDNA strand by nick translation to produce a double stranded cDNA;

5

b) dividing said labeled population into a first portion and a second portion;

c) digesting said first portion with at least one sequence-specific endonuclease selected from the group consisting of at least one endonuclease having a 4 base

10 recognition sequence, at least one endonuclease having a 5 base recognition sequence and  
at least one endonuclease having a six base recognition sequence;

d) digesting said second portion with at least one endonuclease having a  
degenerate recognition sequence wherein said endonuclease produces fragments  
comprising unpaired overhangs containing  $N^m$  unique sequences where N is an integer  
15 between 2 and 4, m is an integer between 2 and 6, and  $N^m$  equals at least 64;

e) isolating nucleic acid fragments from said endonuclease digestions of said first  
and second portions using said end-labels;

f) digesting the fragments of said first portion with said at least one endonuclease  
of d);

20 g) digesting the fragments of said second portion with said at least one  
endonuclease of c);

h) removing labeled nucleic acid fragments from said first and second portions  
while retaining unlabeled fragments;

i) adding a population of adapters, said population containing adapters specific for  
25 at least some of the endonucleases used, wherein each adapter comprises a first region  
specific to a particular endonuclease used and a second region containing a primer  
binding site, said primer binding sites comprising no more than 10 different sequences,  
said sequences lacking significant homology to sequences known to be in said population  
of nucleic acid molecules;

30 j) hybridizing and ligating said adapters to said unlabeled fragments;

k) amplifying said unlabeled fragments using the adapters; and

l) separating the amplified fragments on the basis of size.

29. The method of claim 28, wherein said at least one sequence-specific  
endonuclease comprises at least 4 endonucleases.

30. The method of claim 28, wherein said at least one sequence-specific  
endonuclease comprises at least 6 endonucleases.

31. The method of claim 30, wherein said at least one sequence-specific endonuclease is selected from the group consisting of *Apa* LI, *Bam* HI, *Eco* RI, *Hind* III, *Nco* I, and *Xho* I.

32. The method of claim 28, wherein said at least one endonuclease having a degenerate recognition sequence is *Bsl* I.

33. The method of claim 28, wherein said at least one sequence-specific endonuclease is selected from the group consisting of *Apa* LI, *Bam* HI, *Eco* RI, *Hind* III, *Nco* I, and *Xho* I, and said at least one endonuclease having a degenerate recognition sequence is *Bsl* I.

34. The method of claim 28, where in said adapters comprise a common primer binding site.

35. The method of claim 28, further comprising sequencing the separated fragments of 1).

36. The method of claim 28, further comprising quantifying the separated fragments of 1).

37. The method of claim 28, wherein said label is biotin.

38. The method of claim 28, wherein isolating and removing of end-labeled nucleic acid molecules is by particles that bind said end-labeled nucleic acid molecules.

39. The method of claim 38, wherein said end-label is biotin and said particles comprise avidin or strepavidin.

40. A method for detecting a change in RNA expression in a tissue or cell associated with an internal or external factor comprising, determining RNA expression in a first cell or tissue exposed to the internal or external factor by a method comprising:

- a) obtaining a population of end-labeled double-stranded cDNA molecules from  
5 said first cell or tissue;
- b) dividing said population into a first portion and a second portion;
- c) digesting said first portion with at least one sequence-specific endonuclease;
- d) digesting said second portion with at least one endonuclease having a  
degenerate recognition sequence;
- 10 e) isolating nucleic acid fragments from said endonuclease digestions of said first and second portions using said end-labels;
- f) digesting the fragments of said first portion with said at least one endonuclease of d);
- g) digesting the fragments of said second portion with said at least one  
15 endonuclease of c);
- h) removing labeled nucleic acid fragments from said first and second portions while retaining unlabeled fragments;
- i) adding a population of adapters, said population containing adapters specific for the endonucleases used, wherein each adapter comprises a first region specific to a  
20 particular endonuclease used and a second region containing a primer binding site;
- j) hybridizing and ligating said adapters to said unlabeled fragments;
- k) amplifying the fragments using the adapters;
- l) separating the amplified fragments by size; and
- m) quantitating the separated fragments to produce an RNA expression pattern;
- 25 determining RNA expression in a second cell or tissue of the same type as the first cell or tissue and not exposed to the internal or external factor by repeating a) through m); and comparing RNA expression patterns between the first cell or tissue and the second cell or tissue.

41. The method of claim 40, wherein said tissue or cell is a plant tissue or cell.

42. The method of claim 40, wherein said tissue or cell is an animal tissue or cell.

43. The method of claim 40, wherein said external factor is a disease-causing organism.

44. The method of claim 40, wherein said internal or external factor is a chemical.

45. The method of claim 40, wherein said first cell or tissue is a cancerous cell or tissue.

46. The method of claim 40, wherein RNA expression data for said second tissue is stored on a computer-readable medium, and RNA expression data for said first tissue is compared to the data for said second tissue stored on said computer-readable medium.

47. A method for detecting a change in RNA expression in a tissue or cell associated with an internal or external factor comprising, determining RNA expression in a first cell or tissue exposed to the internal or external factor by a method comprising:

- a) obtaining a population of 3' end-labeled double-stranded cDNA molecules  
5 from said first cell or tissue by a method comprising, isolating polyA mRNA from a cell; hybridizing a 5' end labeled primer containing an oligo dT tail to said polyA mRNA; synthesizing a first cDNA strand by extension of said primer; and synthesizing a second cDNA strand by nick translation to produce a double stranded cDNA;
- b) dividing said population into a first portion and a second portion;
- 10 c) digesting said first portion with at least one sequence-specific endonuclease;
- d) digesting said second portion with at least one endonuclease having a degenerate recognition sequence;
- e) isolating nucleic acid fragments from said endonuclease digestions of said first and second portions using said end-labels;
- 15 f) digesting the fragments of said first portion with said at least one endonuclease of d);



g) digesting the fragments of said second portion with said at least one endonuclease of c);

20 h) removing labeled nucleic acid fragments from said first and second portions while retaining unlabeled fragments;

i) adding a population of adapters, said population containing adapters specific for the endonucleases used, wherein each adapter comprises a first region specific to a particular endonuclease used and a second region containing a primer binding site;

25 j) hybridizing and ligating said adapters to said unlabeled fragments;

k) amplifying the fragments using the adapters;

l) separating the amplified fragments by size; and

30 m) quantitating the separated fragments to produce an RNA expression pattern; determining RNA expression in a second cell or tissue of the same type as the first cell or tissue and not exposed to the internal or external factor by repeating a) through m); and comparing RNA expression patterns between the first cell or tissue and the second cell or tissue.

48. The method of claim 47, wherein said tissue or cell is a plant tissue or cell.

49. The method of claim 47, wherein said tissue or cell is an animal tissue or cell.

50. The method of claim 47, wherein said external factor is a disease-causing organism.

51. The method of claim 47, wherein said internal or external factor is a chemical.

52. The method of claim 47, wherein said first cell or tissue is a cancerous cell or tissue.

53. The method of claim 47, wherein RNA expression data for said second tissue is stored on a computer-readable medium, and RNA expression data for said first tissue is compared to the data for said second tissue stored on said computer-readable medium.

54. A method for detecting a change in RNA expression in a tissue or cell associated with an internal or external factor comprising, determining RNA expression in a first cell or tissue exposed to the internal or external factor by a method comprising:

- a) obtaining a population of 3' end-labeled double-stranded cDNA molecules  
5 from said first cell or tissue by a method comprising, isolating polyA mRNA from a cell; hybridizing a 5' end labeled primer containing an oligo dT tail to said polyA m RNA; synthesizing a first cDNA strand by extension of said primer; and synthesizing a second cDNA strand by nick translation to produce a double stranded cDNA;
- b) dividing said labeled population into a first portion and a second portion;
- 10 c) digesting said first portion with at least one sequence-specific endonuclease selected from the group consisting of at least one endonuclease having a 4 base recognition sequence, at least one endonuclease having a 5 base recognition sequence and at least one endonuclease having a six base recognition sequence;
- d) digesting said second portion with at least one endonuclease having a  
15 degenerate recognition sequence wherein said endonuclease produces fragments comprising unpaired overhangs containing  $N^m$  unique sequences where N is an integer between 2 and 4, m is an integer between 2 and 6, and  $N^m$  equals at least 64;
- e) isolating nucleic acid fragments from said endonuclease digestions of said first and second portions using said end-labels;
- 20 f) digesting the fragments of said first portion with said at least one endonuclease of d);
- g) digesting the fragments of said second portion with said at least one endonuclease of c);
- h) removing labeled nucleic acid fragments from said first and second portions  
25 while retaining unlabeled fragments;
- i) adding a population of adapters, said population containing adapters specific for at least some of the endonucleases used, wherein each adapter comprises a first region

specific to a particular endonuclease used and a second region containing a primer binding site, said primer binding sites comprising no more than 10 different sequences,  
30 said sequences lacking significant homology to sequences known to be in said population of nucleic acid molecules;

j) hybridizing and ligating said adapters to said unlabeled fragments;  
k) amplifying said unlabeled fragments using the adapters;  
l) separating the amplified fragments on the basis of size; and  
35 m) quantitating the separated fragments to produce an RNA expression pattern; determining RNA expression in a second cell or tissue of the same type as the first cell or tissue and not exposed to the internal or external factor by repeating a) through m); and comparing RNA expression patterns between the first cell or tissue and the second cell or tissue.

40

55. The method of claim 54, wherein said tissue or cell is a plant tissue or cell.

56. The method of claim 54, wherein said tissue or cell is an animal tissue or cell.

57. The method of claim 54, wherein said external factor is a disease-causing organism.

58. The method of claim 54, wherein said internal or external factor is a chemical.

59. The method of claim 54, wherein said first cell or tissue is a cancerous cell or tissue.

60. The method of claim 54, wherein RNA expression data for said second tissue is stored on a computer-readable medium, and RNA expression data for said first tissue is compared to the data for said second tissue stored on said computer-readable medium.

61. A method for diagnosing a disease, condition, disorder or predisposition associated with a change in RNA expression comprising determining RNA expression in a first cell or tissue from a subject known to have the disease, condition, disorder or predisposition of interest by a method comprising:

- 5 a) obtaining a population of end-labeled double-stranded cDNA molecules from said first cell or tissue;
- b) dividing said population into a first portion and a second portion;
- c) digesting said first portion with at least one sequence-specific endonuclease;
- d) digesting said second portion with at least one endonuclease having a  
10 degenerate recognition sequence;
- e) isolating nucleic acid fragments from said endonuclease digestions of said first and second portions using said end-labels;
- f) digesting the fragments of said first portion with said at least one endonuclease of d);
- 15 g) digesting the fragments of said second portion with said at least one endonuclease of c);
- h) removing labeled nucleic acid fragments from said first and second portions while retaining unlabeled fragments;
- i) adding a population of adapters, said population containing adapters specific for  
20 the endonucleases used, wherein each adapter comprises a first region specific to a particular endonuclease used and a second region containing a primer binding site;
- j) hybridizing and ligating said adapters to said unlabeled fragments;
- k) amplifying the fragments using the adapters;
- l) separating the amplified fragments by size; and
- 25 m) quantitating the separated fragments to produce an RNA expression pattern; determining RNA expression in a second cell or tissue of the same type from a test subject of the same species by repeating a) through m); and comparing RNA expression from said first cell or tissue to RNA expression from said second cell or tissue.

62. The method of claim 61, wherein said cell or tissue is from a plant.

63. The method of claim 61, wherein said cell or tissue is from an animal.

64. The method of claim 61, wherein said disease, condition, disorder or predisposition is a genetic disease, disorder, condition or predisposition.

65. The method of claim 61, wherein said disease, condition, disorder or predisposition is caused by a microorganism.

66. The method of claim 61, wherein said disease, condition, disorder or predisposition involves cancer.

67. The method of claim 61, wherein RNA expression data for said first cell or tissue is stored on a computer-readable medium, and RNA expression data for said second cell or tissue is compared to the data for said first cell tissue stored on said computer-readable medium.

68. A method for diagnosing a disease, condition, disorder or predisposition associated with a change in RNA expression comprising determining RNA expression in a first cell or tissue from a subject known to have the disease, condition, disorder or predisposition of interest by a method comprising:

- 5       a) obtaining a population of 3' end-labeled double-stranded cDNA molecules from said first cell or tissue by a method comprising, isolating polyA mRNA from a cell; hybridizing a 5' end labeled primer containing an oligo dT tail to said polyA m RNA; synthesizing a first cDNA strand by extension of said primer; and synthesizing a second cDNA strand by nick translation to produce a double stranded cDNA;
- 10       b) dividing said population into a first portion and a second portion;
- c) digesting said first portion with at least one sequence-specific endonuclease;
- d) digesting said second portion with at least one endonuclease having a degenerate recognition sequence;
- e) isolating nucleic acid fragments from said endonuclease digestions of said first
- 15       and second portions using said end-labels;

f) digesting the fragments of said first portion with said at least one endonuclease of d);

g) digesting the fragments of said second portion with said at least one endonuclease of c);

20 h) removing labeled nucleic acid fragments from said first and second portions while retaining unlabeled fragments;

i) adding a population of adapters, said population containing adapters specific for the endonucleases used, wherein each adapter comprises a first region specific to a particular endonuclease used and a second region containing a primer binding site;

25 j) hybridizing and ligating said adapters to said unlabeled fragments;

k) amplifying the fragments using the adapters;

l) separating the amplified fragments by size; and

30 m) quantitating the separated fragments to produce an RNA expression pattern; determining RNA expression in a second cell or tissue of the same type from a test subject of the same species by repeating a) through m); and comparing RNA expression from said first cell or tissue to RNA expression from said second cell or tissue.

69. The method of claim 68, wherein said cell or tissue is from a plant.

70. The method of claim 68, wherein said cell or tissue is from an animal.

71. The method of claim 68, wherein said disease, condition, disorder or predisposition is a genetic disease, disorder, condition or predisposition.

72. The method of claim 68, wherein said disease, condition, disorder or predisposition is caused by a microorganism.

73. The method of claim 68, wherein said disease, condition, disorder or predisposition involves cancer.

74. The method of claim 68, wherein RNA expression data for said first cell or tissue is stored on a computer-readable medium, and RNA expression data for said second cell or tissue is compared to the data for said first cell tissue stored on said computer-readable medium.

75. A method for diagnosing a disease, condition, disorder or predisposition associated with a change in RNA expression comprising determining RNA expression in a first cell or tissue from a subject known to have the disease, condition, disorder or predisposition of interest by a method comprising:

- 5       a) obtaining a population of 3' end-labeled double-stranded cDNA molecules from said first cell or tissue by a method comprising, isolating polyA mRNA from a cell; hybridizing a 5' end labeled primer containing an oligo dT tail to said polyA m RNA; synthesizing a first cDNA strand by extension of said primer; and synthesizing a second cDNA strand by nick translation to produce a double stranded cDNA;
- 10       b) dividing said labeled population into a first portion and a second portion;
- c) digesting said first portion with at least one sequence-specific endonuclease selected from the group consisting of at least one endonuclease having a 4 base recognition sequence, at least one endonuclease having a 5 base recognition sequence and at least one endonuclease having a six base recognition sequence;
- 15       d) digesting said second portion with at least one endonuclease having a degenerate recognition sequence wherein said endonuclease produces fragments comprising unpaired overhangs containing  $N^m$  unique sequences where N is an integer between 2 and 4, m is an integer between 2 and 6, and  $N^m$  equals at least 64;
- e) isolating nucleic acid fragments from said endonuclease digestions of said first
- 20       and second portions using said end-labels;
- f) digesting the fragments of said first portion with said at least one endonuclease of d);
- g) digesting the fragments of said second portion with said at least one endonuclease of c);
- 25       h) removing labeled nucleic acid fragments from said first and second portions while retaining unlabeled fragments;

i) adding a population of adapters, said population containing adapters specific for at least some of the endonucleases used, wherein each adapter comprises a first region specific to a particular endonuclease used and a second region containing a primer  
30 binding site, said primer binding sites comprising no more than 10 different sequences, said sequences lacking significant homology to sequences known to be in said population of nucleic acid molecules;

j) hybridizing and ligating said adapters to said unlabeled fragments;  
k) amplifying said unlabeled fragments using the adapters;  
35 l) separating the amplified fragments on the basis of size; and  
m) quantitating the separated fragments to produce an RNA expression pattern;  
determining RNA expression in a second cell or tissue of the same type from a test subject of the same species by repeating a) through m); and comparing RNA expression from said first cell or tissue to RNA expression from said second cell or tissue.

76. The method of claim 75, wherein said cell or tissue is from a plant.

77. The method of claim 75, wherein said cell or tissue is from an animal.

78. The method of claim 75, wherein said disease, condition, disorder or predisposition is a genetic disease, disorder, condition or predisposition.

79. The method of claim 75, wherein said disease, condition, disorder or predisposition is caused by a microorganism.

80. The method of claim 75, wherein said disease, condition, disorder or predisposition involves cancer.

81. The method of claim 75, wherein RNA expression data for said first cell or tissue is stored on a computer-readable medium, and RNA expression data for said second cell or tissue is compared to the data for said first cell tissue stored on said computer-readable medium.



82. A method for determining the physiological or developmental state of a cell or tissue comprising, determining RNA expression in a first cell or tissue of a known physiological or developmental state by a method comprising:

- 5 a) obtaining a population of end-labeled double-stranded cDNA molecules from said first cell or tissue;
- b) dividing said population into a first portion and a second portion;
- c) digesting said first portion with at least one sequence-specific endonuclease;
- d) digesting said second portion with at least one endonuclease having a degenerate recognition sequence;
- 10 e) isolating nucleic acid fragments from said endonuclease digestions of said first and second portions using said end-labels;
- f) digesting the fragments of said first portion with said at least one endonuclease of d);
- 15 g) digesting the fragments of said second portion with said at least one endonuclease of c);
- h) removing labeled nucleic acid fragments from said first and second portions while retaining unlabeled fragments;
- i) adding a population of adapters, said population containing adapters specific for the endonucleases used, wherein each adapter comprises a first region specific to a particular endonuclease used and a second region containing a primer binding site;
- 20 j) hybridizing and ligating said adapters to said unlabeled fragments;
- k) amplifying the fragments using the adapters;
- l) separating the amplified fragments by size; and
- m) quantitating the separated fragments to produce an RNA expression pattern;
- 25 determining RNA expression in a second cell or tissue from the same species of unknown physiological or developmental state by repeating a) through m); and comparing RNA expression in said first cell or tissue to RNA expression in said second cell or tissue.

83. The method of claim 82, wherein the cell or tissue is from a plant.

84. The method of claim 82, wherein the cell or tissue is from an animal.

85. The method of claim 82, wherein RNA expression data for said first cell or tissue is stored on a computer-readable medium, and RNA expression data for said second cell or tissue is compared to the data for said first cell tissue stored on said computer-readable medium.

86. A method for determining the physiological or developmental state of a cell or tissue comprising, determining RNA expression in a first cell or tissue of a known physiological or developmental state by a method comprising:

- a) obtaining a population of 3' end-labeled double-stranded cDNA molecules  
5 from said first cell or tissue by a method comprising, isolating polyA mRNA from a cell; hybridizing a 5' end labeled primer containing an oligo dT tail to said polyA m RNA; synthesizing a first cDNA strand by extension of said primer; and synthesizing a second cDNA strand by nick translation to produce a double stranded cDNA;
- b) dividing said population into a first portion and a second portion;
- 10 c) digesting said first portion with at least one sequence-specific endonuclease;
- d) digesting said second portion with at least one endonuclease having a degenerate recognition sequence;
- e) isolating nucleic acid fragments from said endonuclease digestions of said first and second portions using said end-labels;
- 15 f) digesting the fragments of said first portion with said at least one endonuclease of d);
- g) digesting the fragments of said second portion with said at least one endonuclease of c);
- h) removing labeled nucleic acid fragments from said first and second portions  
20 while retaining unlabeled fragments;
- i) adding a population of adapters, said population containing adapters specific for the endonucleases used, wherein each adapter comprises a first region specific to a particular endonuclease used and a second region containing a primer binding site;
- j) hybridizing and ligating said adapters to said unlabeled fragments;

- 25 k) amplifying the fragments using the adapters;  
l) separating the amplified fragments by size; and  
m) quantitating the separated fragments to produce an RNA expression pattern;  
determining RNA expression in a second cell or tissue from the same species of unknown  
physiological or developmental state by repeating a) through m); and comparing RNA  
30 expression in said first cell or tissue to RNA expression in said second cell or tissue.

87. The method of claim 86, wherein the cell or tissue is from a plant.

88. The method of claim 86, wherein the cell or tissue is from an animal.

89. The method of claim 86, wherein RNA expression data for said first cell or  
tissue is stored on a computer-readable medium, and RNA expression data for said  
second cell or tissue is compared to the data for said first cell tissue stored on said  
computer-readable medium.

90. A method for determining the physiological or developmental state of a cell  
or tissue comprising, determining RNA expression in a first cell or tissue of a known  
physiological or developmental state by a method comprising:

- a) obtaining a population of 3' end-labeled double-stranded cDNA molecules  
5 from said first cell or tissue by a method comprising, isolating polyA mRNA from a cell;  
hybridizing a 5' end labeled primer containing an oligo dT tail to said polyA m RNA;  
synthesizing a first cDNA strand by extension of said primer; and synthesizing a second  
cDNA strand by nick translation to produce a double stranded cDNA;  
b) dividing said labeled population into a first portion and a second portion;  
10 c) digesting said first portion with at least one sequence-specific endonuclease  
selected from the group consisting of at least one endonuclease having a 4 base  
recognition sequence, at least one endonuclease having a 5 base recognition sequence and  
at least one endonuclease having a six base recognition sequence;  
d) digesting said second portion with at least one endonuclease having a  
15 degenerate recognition sequence wherein said endonuclease produces fragments

comprising unpaired overhangs containing  $N^m$  unique sequences where N is an integer between 2 and 4, m is an integer between 2 and 6, and  $N^m$  equals at least 64;

e) isolating nucleic acid fragments from said endonuclease digestions of said first and second portions using said end-labels;

20 f) digesting the fragments of said first portion with said at least one endonuclease of d);

g) digesting the fragments of said second portion with said at least one endonuclease of c);

25 h) removing labeled nucleic acid fragments from said first and second portions while retaining unlabeled fragments;

30 i) adding a population of adapters, said population containing adapters specific for at least some of the endonucleases used, wherein each adapter comprises a first region specific to a particular endonuclease used and a second region containing a primer binding site, said primer binding sites comprising no more than 10 different sequences, said sequences lacking significant homology to sequences known to be in said population of nucleic acid molecules;

j) hybridizing and ligating said adapters to said unlabeled fragments;

k) amplifying said unlabeled fragments using the adapters;

l) separating the amplified fragments on the basis of size; and

35 m) quantitating the separated fragments to produce an RNA expression pattern; determining RNA expression in a second cell or tissue from the same species of unknown physiological or developmental state by repeating a) through m); and comparing RNA expression in said first cell or tissue to RNA expression in said second cell or tissue.

91. The method of claim 90, wherein the cell or tissue is from a plant.

92. The method of claim 90, wherein the cell or tissue is from an animal.

93. The method of claim 90, wherein RNA expression data for said first cell or tissue is stored on a computer-readable medium, and RNA expression data for said

second cell or tissue is compared to the data for said first cell tissue stored on said computer-readable medium.

94. A computer readable medium having stored thereon computer executable instructions for performing a method comprising, receiving data on RNA expression from a first cell or tissue, said data produced by the method of claim 1; and comparing said data from said first tissue with RNA expression data produced by the method of claim 1  
5 from a second cell or tissue of the same species as said first cell or tissue.

95. A method for constructing a nucleic acid library comprising:

a) obtaining a population of double stranded cDNA wherein cDNA molecules contained in said population contain a detectable label on their 3' end;

b) digesting said double-stranded cDNA with at least one restriction endonuclease  
5 having a degenerate recognition sequence comprising at least one degenerate base, wherein said digestion creates a single-stranded portion or overhang containing a region having the formula  $N^m$ , where N is the extent of degeneracy and m is the number of degenerate bases in said single stranded portion or overhang to produce digestion fragments;

10 c) adding a population of adapters, said adapters specific for the at least one endonuclease used;

d) hybridizing and ligating said adapters to the 5' end of said digestions fragments;

e) separating said 3' end digestion fragments using said detectable label;

15 f) amplifying said 3' end digestion fragments;

g) separating said amplified digestion fragments on the basis of size.

96. The method of claim 95, wherein said adapters contain first region specific to a particular endonuclease and a second primer binding region common to all adapters.

97. The method of claim 95 wherein  $N^m$  equals at least 16.

TM0066-UT  
Patent

98. The method of claim 97, wherein said at least one endonuclease is *BsaJ* I.

[illegible]